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Hereditary breast cancer is frequently due to a germline heterozygous mutation of either Brca1 or Brca2. However, familial breast cancer accounts for only a small minority of breast cancer cases. Sporadic breast cancer, on the other hand, represents 90% of breast cancer patients. Mutations of both oncogenes and tumor suppressor genes often occur in spontaneous breast cancer. Specifically, tumor suppressor gene activity may be abrogated or decreased in cancer cells. Recently, a putative tumor suppressor gene, *DBC2* (Deleted in Breast Cancer), was discovered that appears to be frequently mutated in sporadic breast cancer. *DBC2* is suspected to be a tumor suppressor gene important for breast cancer because: 1) *DBC2* expression cannot be detected in half of the spontaneous breast cancer tissues and cells tested and 2) wild-type (WT) *DBC2* expressed in a breast cancer cell line, T47D, inhibited cellular proliferation while mutated *DBC2* expression did not repress growth of the breast cancer cells. These data imply that mutation of DBC2 is important for the development of spontaneous breast cancer. This proposal serves to investigate the functional role of DBC2 in cells and mice to elucidate the function of DBC2 for tumor suppression. To this end, we have generated embryonic stem cells heterozygous for *DBC2* to execute these studies.

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A Mouse Model to Investigate the Role of *DBC2* in Breast Cancer

INTRODUCTION

Breast cancer is a major cause of mortality among women. The American Cancer Society predicts that about one in eight women will be diagnosed with breast cancer (3). Deletion of tumor suppressor genes play an important role in both familial and sporadic breast cancer (1). Hereditary breast cancer is frequently due to a germline heterozygous mutation of either Brca1 or Brca2 (4). Familial breast cancer represents only 10% of total breast cancer cases; and Brca2 is not mutated for spontaneous breast cancer; thus, the etiology for 90% of breast cancer is largely unknown. However, Hamaguchi and colleagues recently discovered a putative tumor suppressor gene, *DBC2* (deleted in breast cancer), that appears to be frequently mutated in sporadic breast cancer (2). The *DBC2* gene lies within a region of human chromosome 8p21. The putative functional domains of *DBC2* include a RAS domain and two protein-protein interaction domains called the BTB/POZ domains. *DBC2* is suspected to be a tumor suppressor gene important for breast cancer because: 1) *DBC2* expression cannot be detected in half of the spontaneous breast cancer tissues and cells tested and 2) wild-type (WT) *DBC2* expressed in a breast cancer cell line, T47D, inhibited cellular proliferation while mutated *DBC2* expression did not repress growth of the breast cancer cells. These data imply that mutation of *DBC2* is important for the development of spontaneous breast cancer. Furthermore, recent microarray data shows *DBC2* to have an influence on the following pathways: cell-cycle, apoptosis, cytoskeleton, and membrane-trafficking; suggesting a role for *DBC2* in carcinogenesis (5). However, due to the fact that *DBC2* was just recently discovered, little is known about its function. This work serves to investigate the functional role of *DBC2* in cells and mice to elucidate the function of *DBC2* for tumor suppression.

BODY

Specific Aim 1: Characterize the phenotype of *DBC2*-mutant mouse ES cell and MEF clones

a) Generate *DBC2*-mutant ES cells
b) Generate knock-ins of wild type and altered *DBC2* cDNAs in ES cells
c) Perform a genotoxic screen on wild type and knock-in ES cells

d) Analyze cell cycle checkpoints and apoptosis in knockout MEF

In the past year, I have constructed a targeting vector to mutate *DBC2* in ES cells. Importantly, a positive selection cassette replaces the initiating ATG and some surrounding sequence. The vector was electroporated into ES cells and upon selection I successfully obtained three targeted clones (Fig. 1). Also shown in Fig. 2 is the screening strategy that utilizes RT-PCR.

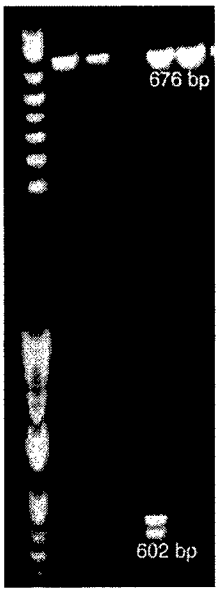
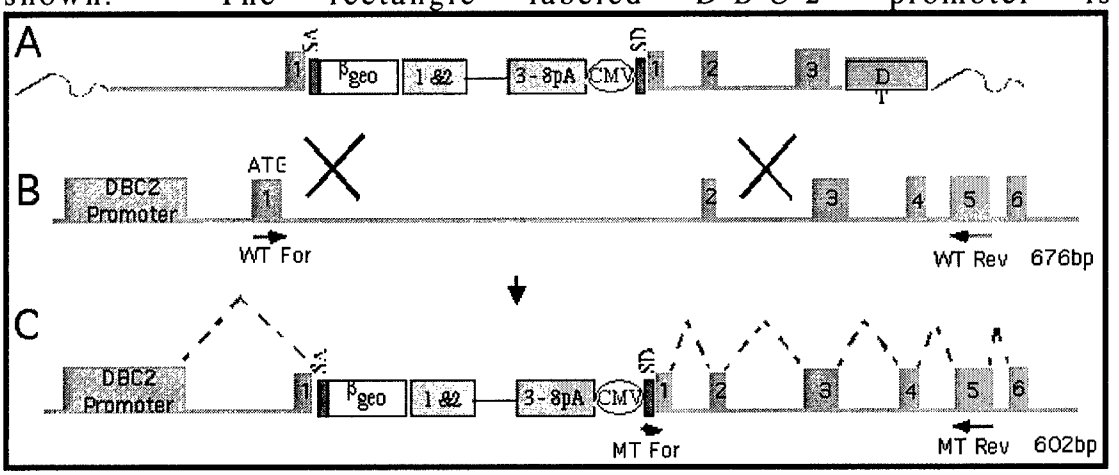


Figure 1. Ethidium Bromide stained agarose gel; 676bp product represents wildtype *DBC2*; 602bp product is the targeted mutant allele.

To our knowledge, this is the first time such a method has been used to screen for targeted ES cell clones. This analysis included extracting RNA from the potential targeted ES cell cones, the generation of cDNA, and PCR amplification of the region of interest. Fig. 2 depicts the location of the primers used to amplify both wildtype and mutant *DBC2*. The forward primer used to amplify the wildtype band is situated in exon 1 containing the initiation ATG, the region deleted in the mutant allele (Fig. 2B). Alternatively, the mutant forward primer is positioned 3' to the CMV promoter of the selection cassette in an artificial exon (Fig. 2C). Therefore, this primer should only anneal in a clone targeted for *DBC2*. Both the wildtype and mutant alleles utilize the same reverse primer located in a downstream exon.

Figure 2. A. Gene targeting vector: deletes the translation initiation ATG with a promoterless Neo and an HPRT minigene. **B**. *DBC2* genomic locus. Six exons are shown. The rectangle labeled *DBC2* promoter is



undefined and presumed to be located 5' to the exon that contains the translation initiation ATG. WT For and WT Rev show the location of the primers used to identify wildtype *DBC2* by RT-PCR screening. **C**. Insertion of selection cassette into *DBC2*

gene. Part of exon 1 is deleted such that the selection cassette replaces the translation initiation ATG. MT For and MT Rev show the location of the primers used to identify the mutant *DBC2* by RT-PCR (Fig. 2B,C)

Furthermore, the amplified sequence from the potential target was sequenced to confirm targeting using the MT For and MT Rev primers. The sequencing results show the allele is targeted. In addition, I have constructed another *DBC2* targeting vector containing a Puromycin selection cassette. This vector will be used to target the other *DBC2* allele of the targeted clone.

Finally, the ES cells heterozygous for *DBC2* are being expanded and will be used for microinjection into mice.

Specific Aim 2: Generate and analyze *DBC2* knockout mice

DBC2 knockout mice will be generated and analyzed throughout their entire life span with particular attention given to cancer onset, incidence and spectrum. In addition, in order to investigate any potential tumor suppressor function *DBC2* has for mammary carcinoma in mice, the *DBC2*-mutation will be crossed into transgenic mice predisposed to mammary carcinoma that contain a *neu* proto-oncogene and a dominant negative p53 transgene. The mutant mice will be studied in cohorts of 30 mice (30 *DBC2*+/+, 30 *DBC2*+/-, and 30 *DBC2*-/-).

We are currently preparing the $DBC2^{+/-}$ ES cells for injection into blastocysts.

KEY RESEARCH ACCOMPLISHMENTS

- Generation of $DBC2^{+/-}$ ES cells
- Development of RT-PCR assay to detect targeted ES cells

REPORTABLE OUTCOMES

Abstracts:

None.

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None

Awards:

None.

CONCLUSIONS

During this past year, we have obtained mouse embryonic stem cells targeted for *DBC2*. We have also developed a novel method of screening ES cell clones for targeted alleles using RT-PCR. Additionally, we are preparing to begin microinjections with the targeted ES cells to obtain mice mutant for the *DBC2* gene. These mice will be generated and analyzed throughout their entire life span with particular attention given to cancer onset, incidence and spectrum. At the same time, we will soon begin a second round of gene targeting to acquire homozygous mutant ES cells. We will perform a genotoxic

screen on the mutant cells to help elucidate a function for *DBC2* in the ES cells by testing multiple pathways important for chromosomal metabolism and responses to DNA alterations. Our studies will address the novel functional activity of *DBC2* that could have a large impact in our understanding of spontaneous breast cancer development. It is our hope that these studies will help to understand the putative activity of *DBC2* in the cell and in the mouse. The *DBC2*-mutant mice may also provide a mouse model for spontaneous breast cancer and elucidate the importance of *DBC2* as a tumor suppressor. This discovery could open many new doors for the development and implementation of drugs for the treatment of breast cancer.

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